



DKT. 13131

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**Applicant: Susan L. Weston *et al.*

Art Unit: 1655

Serial No.: 09/228,639

Examiner: Enewold, J.

Date Filed: 12 January 1999

Docket No.: 13131

For: SEQUENCES

September 10, 2002

Hon. Commissioner of Patents and Trademarks  
Washington, D.C. 20231

**SUPPLEMENTAL DECLARATION**

Sir:

1. I, Dr. Gary Brown, am skilled in the art of Molecular Biology. I am a scientific consultant in biotechnology and am being compensated for making this declaration.
2. I make this declaration based upon my training, knowledge, education, and experience as a molecular biologist, my review of the application and the history of the prosecution of this application as reflected in the file maintained by the attorneys prosecuting this application, and my review of prior art cited by the Examiner during prosecution of this application, and my review of inventor laboratory monthly project reports (laboratory reports) and the CF 12 Test Prototype Record Sheets (record sheets) attached as Exhibit D.
3. In the March 12, 2002 Office Action, the Examiner states on page 3 that "[T]he exhibits of the laboratory reports however do not appear to identify primers by either

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sequence or by SEQ ID NO: as relied upon in the instant specification. The exhibits are general to primers designated by mutational name and do not provide any information as to the structure of the primers which fail. Therefore, it is unclear whether these primers which failed are the primers claimed or whether they are within the scope of the claims or whether they constitute unexpected results. Further, the declaration asserts that primer length affected the results of the assay. As explained above, while the declaration asserts primers of different lengths were used, it is not clear from the manual or from the declaration which of these lengths was ideal and whether this result is within the scope of the claims."

4. In this declaration, I provide the record sheets that I reviewed, which identifies, among other things, some of the primer sequences used by name, changes made to primer sequences, nucleotide lengths and concentrations.
5. Based upon my review of, among other things, the laboratory reports (previously submitted as Exhibits B and C) of Susan Weston (Susie Weston) and the record sheets, as one skilled in the art, I conclude that the laboratory reports and record sheets detail the unexpected results of the invention. The reports and record sheets record difficulties, the multiple failures, and the surprising results of the multiple experiments performed by the inventor in the present application. These experiments show unexpected results after modifications of numerous parameters of the experimental system.
6. Inventor Susie Weston, in her laboratory report from Lab books NBY 5882 and NBY.

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5922, and record sheets shows that certain primers were prone to non-specific priming which makes such primers unacceptable for use in this assay. A number of unacceptable primer dimer results were observed, and a reversed direction primer was selected and introduced as a standard, to avoid primer dimers (Exhibit B, page 1). In addition, zinc concentrations and temperature were varied. Some of the unacceptable results the inventor documented were weak signal, very weak signal, primer dimer, and nonspecific bands (Exhibit B, page 2).

7. In further experiments, inventor Susie Weston designed primers (see for example, record sheets documenting changes to prototype 2 and prototype 3, involving primers: KRT 16, R334WM, R1162XM and R1162XC), varied concentrations of primers, tried zinc-free preparations, and varied the annealing times. Results of these modifications that the inventor recorded included unacceptably faint signals and smeared products, absent product, and nonspecific products (Exhibit B, page 3). For example, the inventor found that primer R553XM (see prototype 1 of the record sheets) did not perform as expected and changed the 3' sequence of the primer from CCA to TGA. The changed sequence was found to work well in conjunction with the other primers and was introduced as a standard in the set and is now designated as SEQ ID No. 24. Further the inventor found the primers KRT 16 reverse, R334WC forward and R1162XM reverse (see prototype 2 of the record sheets) did not function and were changed dramatically. For example, in Exhibit B the inventor states "[t]aking into account that the changes in mixes 2 and 3 had no adverse effect and were potentially beneficial the new 621+1/R117HC, 0.5 $\mu$  M R334WC, R1162XM and R1162WC and 2 $\mu$  M621+1M and R553XM were introduced as standard." These primers introduced

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as standard are named: R334WC (SEQ ID No:22), R1162XM (SEQ ID NO: 26) and R553XM (SEQ ID No: 24). Primer 621+1/R117HC was changed when it failed to work as expected in the final stages of prototype testing (see last prototype CF(12)m Test: Prototype Record Sheet B Tube [P8] of record sheets). This new 621+1/R117HC primer is now (SEQ IN No: 20). Interestingly, the inventor later found the new R1162WC surprisingly did not function well (see Exhibit C, first page, last paragraph) and reinstated primer R1162XC as standard now SEQ ID NO: 27. Accordingly, one of ordinary skill in the art can easily see from the sheer number of modifications to the assays (primer sequence and chemical modifications) that the inventor did not simply optimize a functioning assay. Rather, the inventor had to discover an assay and all of its components by changing primer sequences, primer concentrations and chemical components of the assay in a way that was not routine to achieve the claimed invention. Thus, as can be seen in the specification, notebook pages and record sheets, I conclude that this inventor designed multiple variations of the experiment expecting to overcome problems of nonspecificity, weak products, and primer dimer contaminants, she observed unexpected results and had to discover and assay that worked by numerous trials, drawing on further variations and further experiments that were not routine to achieve the claimed invention.

8. Inventor Susie Weston, in her laboratory report from Lab books NBY 5922 and NBY 5935 (Exhibit C) concludes that the results she obtained from further experiments were unexpected and were not routine. Following experiments that attempted to eliminate nonspecific products and increase band intensities—problems from the previous months' results—some resulting signals were again too weak, and so she

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varied the concentrations of primers. She also varied primer length, using 29mers, 26mers, 24mers, and also temperature changes in the protocol as well (Exhibit C, page 1). I particularly note the following observation of the R1162XC primer (see primer sequence on prototype 3 record sheet) by her: **"This surprisingly had the reverse effect -all ...DNAs were specific with the original primer and non-specific with the new primer"** (Exhibit C, page 1, emphasis added). She went on to vary concentration of primers, lengths of primer, dropped certain primers, varied annealing temperatures, varied Taq enzyme units, and varied DNA concentrations (Exhibit C, page 2). Results she described were weak, very weak, nonspecific—i.e. unacceptable.

9. Inventor Susie Weston, in her laboratory report from Lab books NBY 5935 and NBY 6107, shows her experimental variation of dNTP reagent concentration, and design of new primers to attempt to overcome mispriming difficulties. Primer length and identity of primers were varied. Her results included nonspecific bands, weak diagnostics, and extremely weak non-specific bands (Exhibit C, page 3). A result noted by the inventor states **"[i]t was thought it might come up 621+1 as only the 3' base would be mismatched but much to our relief it didn't."** (Exhibit C, page 3, paragraph 3). Nonspecific signals and weak signals continued to be problematic, which shows that even more experimentation was needed. Accordingly, I conclude that the inventors modifications sometimes resulted in improved assays other times resulted in new or continued problems, and that these results to obtained the presently disclosed primers were not routine.

10. At page 3 of the March 12, 2002 Office Action, the Examiner states: "[a]s noted in *In*

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*re Aller*, 105 USPQ 233 at 235, more particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." The Examiner further states that routine optimization is not considered inventive and no evidence has been presented that primer selection performed was other than routine, that the products resulted from optimization have any unexpected properties, or the results should be considered unexpected in any way as compared to the closest prior art.

11. I respectfully disagree with the Examiner. None of the cited references relied upon by the Examiner disclose the primer sets of the claimed invention that allow optimization and production of meaningful results in the ARMS assay. In my opinion, the presently claimed primer sets were not known prior to the instant invention, and it is not routine optimization to select these primer sets out of the numerous primers that did not work and get them to function in the ARMS assay. As shown by the specification, the exhibits submitted and the declarations, Applicants did not simply optimize a known set of primers or ARMS parameters. Applicants discovered how to design primers of the instant invention and establish conditions to allow those primers to function together simultaneously in primer sets to yield reproducible and valid results. Accordingly, this was not routine experimentation.
12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001

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of Title 18 of the United States Code and that such willful false statements may  
jeopardize the validity of the application or any patent issued thereon.

Gary L. Brown  
Dr. Gary L. Brown

9-11-02  
Date



ZENECA Diagnostics

## F12 Test: Prototype Record Sheet

Tube B Prototype 1 Operator SUSIE Date            Standard ARMS/dNTP? YES

Modifications since last prototype           

Primer	F/R	Oligo ID	Mismatch	Length	Concn (µM)	Sequence
AP0B	F	004795	C-A-2	29	0.25	CTCTGGGAGCACAGTACGAAAAACCAC CT
AP0B	R	004895	C-A-2	30	0.25	CAGCCAAAACCTTTTACAGGGATGGAGAACGT
KRT16	F	14990	C-T-2	28	0.1	CTGAGCACCTTTCTCTCTTTCAGCCACC
KRT16	R	14991	A-C-2	28	0.1	CATGGAGCTGGAGGAGGTGAACCTGGCAG
ΔFN	R	001794	C-T-2	30	1	GTATCTATATTTCATCATAGGAAACACC ACA
ΔFC	F	001894	—	30	1	GACTTCACTTCTAATGATGATTATGGGAGA
62H1M	R	2671	G-T-2	30	1	TGCCATGGGGCCTGTGCAAGGAAGTAT TGA
R117H1M	R	245411	C-C-2	30	1	AGCCTATGCCTAGATAAATCGCGATAG ACT
621R117H1M	F	2678P	—	30	1	TCACATATGGTATGACCCCTCTATATAA ACT
R334WM	R	245402	A-G-2	30	1	GCAGAAAGAGATGGTGGTGAATATTTT CAA
R334WC	F	245404	—	30	1	GAAGGCAGCCTATGTGAGATACTTCAATAG
G551DM	R	002094	T-T-2	25	1	GCTAAAGAAATTCTTGCTCGTTGTT
R553XM	R	001694	C-A-3	60	1	GACTGACTGACTGACTGACTCTGACTGACTTAT
				nhtail		TCACCTTGCTAAAGAAATCTTGCCCA
G551P553X	F	004395	—	28	1	TAAAATTTTCAGCAATGTTGTTTTT GACC
R1162XM	R	245409	A-G-2	30	1	TGTTGGCATGTCAATGAACTTAAAGACTAA
R1162XC	F	245405	—	30	1	AATTATTTCTCTGTTAGTTCATTGAAAAGCC

### Mixes sent out:

Process Number	Quantity	Destination	Comments
4145-06	60	Mike W.	sent <u>          </u>
			94°C-1', 58°C-1', 72°C-1' x 35. 2uTag / test.
4145-06	60	Rich	sent <u>          </u>
			94°C-1', 58°C-1', 72°C-1' x 35 2uTag / test.

### Other Information:

cf12prot.sam



# **CF12 Test: Prototype Record Sheet**

Tube B Prototype 2 Operator SUSIE Date [REDACTED] Standard ARMS/dNTP? YES

Modifications since last prototype [APOB], [KRT], R553XM mismatch

Primer	F/R	Oligo ID	Mismatch	Length	Concn (µM)	Sequence
APOB	F	0047.95	C-A-2	29	0.1	CTCTGGGAGCACAGTACCTAATAACACCT
APOB	R	0048.95	C-A-2	30	0.1	CAGCCAAACTTTTACAGGGATGGAGAACG
KRT16	F	M4990	C-T-2	28	0.25	CTGAGCACCTTCTCTTTCAGCCACC
KRT16	R	M4991	A-C-2	28	0.25	CATGGAGCTGGAGGAGGTGAAGTGGCA
ΔFN	R	0017.94	C-T-2	30	1	GTATCTATATTCATCATAGGAAACACCACA
ΔFC	F	0018.94	—	30	1	GACTTCACTTCTAATGATGATTATGGGAGA
621+1M	R	2671	G-T-2	30	1	TGCCATGGGGGCTGTGCAAGGAAGTATGA
R117+1M	R	24541	C-C-2	30	1	AGCCTATGCCCTAGATAAATCGCGATAGACT
621/R17H	F	2678P	—	30	1	TACATATGGTATGACCTCTATATAAACT
R334WM	R	245402	A-G-2	30	1	GCAGAAATGAGATGGTGGTGAATATTTTCAA
R334WC	F	245404	—	30	1	GAAGGCAGCCTATGTGAGATACTTCAATAG
G551DM	R	0020.94	T-T-2	25	1	GCTAAAGAAATTCCTTGCTCGTTGTT
R553XM	R	2235	G-G-2	60	1	GACTGACTGACTGACTGACTCTGACTGACT
				nhkaii		TATTCACCTTGCTTAAGAAATTCCTTGCTGA
G551/R553X	F	0043.95	—	28	1	TAAATTCAGCAATGTTGTTTIGACC
R1162XM	R	245409	A-G-2	30	1	TGTTGGCATGTCAATGAACTTAAGACATA
R1162XC	F	245405	—	30	1	AATTATTTCTGTAGTTTATGAAAAATGCC

## **Mixes sent out:**

Process Number	Quantity	Destination	Comments
2175-03	?	Mike W.	sent [REDACTED]
			94°C-1', 59°C-1', 72°C-30sec x 35 2UTAG/KSL
4185-03	144	Mike W.	sent [REDACTED]
			94°C-1', 58°C-1', 72°C-1' x 35 2UTAG/KSL

## **Other Information:**

4185-03 contained 2 repeat primer syntheses:  
0017-94 replaced by 0058-95

cf12prot.sam 0018-94 replaced by 0059-95



# **CF12 Test: Prototype Record Sheet**

Tube B Prototype 4 Operator SUSIE Date [REDACTED] Standard ARMS/dNTP? Yes

Modifications since last prototype shorter Apo B primers, dec. [ΔF], dec. [R1162X]  
dec. [R334W], inc. [G551D/R553X], inc. [G21+1/R117H]  
inc. [KRT]

Primer	F/R	Oligo ID	Mismatch	Length	Concn (μM)	Sequence
APO B	F	0197-95	C-A-2	24	0.1	GGAGCACAGTACGAAAAACCACT
APOB	R	0198-95	C-A-2	26	0.1	CAAAACTTTTACAGGGATGGAGAA CG
KRT16	F	0161-95	C-T-2	28	0.3	CTGAGCACCTTTCCTTCTTTCAGCCAC C
KRT16	R	0162-95	A-C-2	27	0.3	TTTATGGAGCTGGAGGAGGTGA ACT
ΔFN	R	0176-95	C-T-2	30	0.5	GTATCTATATTCATCATAGGAAACACC ACA
ΔFC	F	0177-95	—	30	0.5	GACTTCACTTCTAATGATGATTATGGG AGA
G21+1M	R	0178-95	G-T-2	30	2	TGCCATGGGGGCTGTGCAAGGAAG TATTGA
R117HM	R	0179-95	C-C-2	30	1	AGCCTATGCTAGATAAATCGCGATAG ACT
G21/R117H C	F	0196-95	<sup>C-T-2</sup> <sub>T-A-2</sub>	34	1.5	TCACATAGTGTA TGACCCCTATATACACT CATT
R334WM	F	P1337	G-G-2	30	0.25	CCTATGCACTAATCAAAGGAATCATCC TGT
R334WC	R	0192-95	—	30	0.25	TTTGTTTATTGCTCCAAGAGAGTCATA CCA
G551DM	R	0190-95	T-T-2	25	1	GCTAAAGAAATTCTTGCTGGTTGTT
R553XM	R	0191-95	G-G-2	60	2	GACTGACTGACTGACTGACTCTGACTGACT TAT
			nh tail			TCACCTTGCTAAAGAAATTCTTGCTGA
G551D/R553X C	F	0193-95	<sup>G-G-2C</sup> <sub>G-A-21</sub>	28	1.5	TAAATTGGAGCAATGTTGTTTGTGAC C
R1162XM	F	0157-95	G-T-2	30	0.25	TATTTTATTTCAGATGCGATCTGTGA GTT
R1162XC	R	P5732	—	29	0.25	TTT.TGCTGTGAGATCTTTGACAGTCA TTT

## **Mixes sent out:**

Process Number	Quantity	Destination	Comments
1395-01	200+	Nikki D.	sent [REDACTED]
1395-01	22	Nikki D.	sent [REDACTED] to evaluate alkali + phenol extraction of CF bloods
1395-01	50	RMCH	sent [REDACTED]
1395-01	20	Nikki D.	sent [REDACTED]

## **Other Information:**

Cycling: 94°C-1', 58°C-2', 72°C-1'  
 2 Utaq/reaction. Recommended 50ng DNA/reaction.

cf12prot.sam

## CF12 Test: Prototype Record Sheet

Tube B Prototype 5 Operator Susie Date [redacted] Standard ARMS/dNTP? yesModifications since last prototype ODC controls, dec. [APOB]

Primer	F/R	Oligo ID	Mismatch	Length	Concn ( $\mu$ M)	Sequence
APOB	F	0197.95	C-A-2	24	0.075	GGAGCACAGTACGAAAAACCCACT
APOB	R	0198.95	C-A-2	26	0.075	CAAACTTTTACAGGGATGGAGAACC
ODC	F	0235.95	C-T-2	30	0.3	AGAGGATTATCTATGCAATCCTTGTA
ODC	R	0236.95	A-C-2	30	0.3	TCAACTTCACTATCAAAAGTCATC
$\Delta$ FN	R	0176.95	C-T-2	30	0.5	GTATCTATATTCATCATAGGAAACALL
$\Delta$ FC	F	0177.95	—	30	0.5	GACTTCACTTCTAATGATGATTATGGG
62H1M	R	0178.95	G-T-2	30	2	TGCCATGGGGCTGTGCAAGGAAGTATTGA
R117HM	R	0179.95	C-C-2	30	1	AGCCTATGCTTAGATAAAATCGCGATAGACT
62H1M	F	0196.95	<sup>5'-T-36</sup> <sub>3'-A-37</sub>	34	1.5	TCACATAGTGTATGACCTCTATATACACTCATT
R334WM	F	P1337	G-G-2	30	0.25	CCTATGCACTAATCAAAAGGAATCATCTCT
R334WC	R	0192.95	—	30	0.25	TTTGTATTATGCTCCAAGAGAGTCAATCCA
GSSIDM	R	0190.95	T-T-2	25	1	GCTAAAGAAATTCCTTGCTCGTTGTT
R553XM	R	0191.95	G-G-2	60	2	GACTGACTGACTGACTGACTCTGACTGACT
			nkdil			TATTCACCTTGCTAAAGAAATTCCTTGCTGA
GSSIDM	F	0193.95	<sup>5'-G-20</sup> <sub>3'-A-21</sub>	28	1.5	TAAATTTGGAGCAATGTTGTTTGGACC
R1162XM	F	0157.95	G-T-2	30	0.25	TATTTTATTTTCAGATGCGATCTGTGAGTT
R1162XC	R	P5732	—	29	0.25	TTTGTCTGTGAGATCTTTGACAGTCATTT

## Mixes sent out:

Process Number	Quantity	Destination	Comments
4465-02	53	Nikki D.	Box 4 for 50 mouthwashes, sent [redacted]
3435-04	120	KG centre	took [redacted]
3495-04	55	Nikki D.	for 50 mouthwashes sent [redacted]
			using fresh control primer syntheses.

contaminated  
: 15/02/04

## Other Information:

Cycling: 94°C-1', 58°C-2', 72°C-1' x 35  
2 uTag/reaction

cf12prot.sam

50 ng DNA/reaction or alk. prep.

# **CF12 Test: Prototype Record Sheet**

Tube B Prototype 6 Operator SUSIE Date            Standard ARMS/dNTP? YES

Modifications since last prototype even shorter 'ApoB3' (at 100 concn)  
dec. [R117HM], [R1162XM], [R1162XC], [R334WM], [R334WC]

Primer	F/R	Oligo ID	Mismatch	Length	Concn (µM)	Sequence
ApoB	F	0304-95	C-A-2	22	0.1	AGC ACA GTA CGA AAA ACC ACC T
ApoB	R	0305-95	C-A-2	22	0.1	ACT TTTACAGGG ATG GAG AAC G
ODC	F	0302-95	C-T-2	30	0.3	AGAGG ATTATC TATGCAAATCCTTGTA ACC
ODC	R	0303-95	A-C-2	30	0.3	TCAACTTCACTATCAAAAGTCATCATCTAG
ΔFN	R	0176-95	C-T-2	30	0.5	G TATCTATATTCATCATAGGAAACACC. ACA
ΔFC	F	0177-95	—	30	0.5	GACTTCAC TTCTAATGATGATTATGGG AGA
Q21M	R	0178-95	G-T-2	30	2	TGCCATGGGGCCTGTGCAAGGAAGTATTGA
R117HM	R	0179-95	C-C-2	30	0.5	AGCCTATGCCTAGATAAATGCGATAG ACT
Q21/R117M	F	0196-95	<sup>E-T-B</sup> <sub>A-C-34</sub>	34	1.5	TCACATAGTGTATGACCTCTATATACACTCAT T
R334WM	F	P1337	G-G-2	30	0.2	CCTATGCACTAATCAAAGGAATCATCCTGT
R334WC	R	0192-95	—	30	0.2	TTTGTTTATTGCTCCAAGAGAGTCATACCA
G551DM	R	0190-95	T-T-2	25	1	GCTAAAGAAATTCCTGCTCGTTGTT
R553XM	R	0301-95	G-G-2	60	2	GACTGACTGACTGACTGACTCTGACTGACTTAT
—	—	—	—	ntail	—	TCACCTTGCTAAAGAAATTCCTGCTGA
G551DM/R553X	F	0193-95	<sup>G-G-20</sup> <sub>A-A-21</sub>	28	1.5	TAAAATTGGAGCAATGTTGTTTGTGACC
R1162XM	F	0157-95	G-T-2	30	0.2	TATTTTATTTCAGATGCGATCTGTGAGTT
R1162XC	R	P5732	—	29	0.2	TTTGCTGTGAGATCTTTGACAGTCATTT

## **Mixes sent out:**

Process Number	Quantity	Destination	Comments

## **Other Information:**

now 2.5 uTag / reaction

# CF(12)m Test: Prototype Record Sheet

## B Tube [P7]

1x ARMS, 100µM dNTPs

Modifications since last prototype:

new ApoB primers, each at 0.05µM

Primer	F/R	Oligo ID	Mismatch	Length	Concn (µM)	Sequence
Apo B	F	S5890	CA -2	23	0.05	GAG CAC AGT ACG AAA AAC CAC CT
Apo B	R	S5891	CA -2	24	0.05	AAA CTT TTA CAG GGA TGG AGA ACG
ODC	F	0302-95	CT -2	30	0.30	AGA GGA TTA TCT ATG CAA ATC CTT GTA ACC
ODC	R	0303-95	AC -2	30	0.30	TCA ACT TCA CTA TCA AAA GTC ATC ATC TAG
DF N	R	0178-95	CT -2	30	0.50	GTA TCT ATA TTC ATC ATA GGA AAC ACC ACA
DF C	F	0177-95	-	30	0.50	GAC TTC ACT TCT AAT GAT GAT TAT GGG AGA
621 M	R	0178-95	GT -2	30	2.00	TGC CAT GGG GCC TGT GCA AGG AAG TAT TGA
R117H M	R	0179-95	CC -2	30	0.50	AGC CTA TGC CTA GAT AAA TCG CGA TAG ACT
621/R117H C	F	0196-95	CT -8 TC -26 GA -27	34	1.50	TCA CAT AGT GTA TGA CCC TCT ATA TAC ACT CAT T
R334W M	F	P1337	GG -2	30	0.20	CCT ATG CAC TAA TCA AAG GAA TCA TCC TGT
R334W C	R	0192-95	-	30	0.20	TTT GTT TAT TGC TCC AAG AGA GTC ATA CCA
G551D M	R	0190-95	TT -2	25	1.00	GCT AAA GAA ATT CTT GCT CGT TGT T
R553X M	R	0301-95	GG -2	60	2.00	GAC TGA CTG ACT GAC TGA CTC TGA CTG ACT TAT TCA CCT TGC TAA AGA AAT TCT TGC TGA
G551D/R553X C	F	0193-95	GG -20 GA -21	28	1.50	TAA AAT TGG AGC AAT GTT GTT TTT GAC C
R1162X M	F	0300-95	GT -2	30	0.20	TAT TTT TAT TTC AGA TGC GAT CTG TGA GTT
R1162X C	R	P5732	-	29	0.20	TTT TGC TGT GAG ATC TTT GAC AGT CAT TT

Operator

Sirle Weston

Date

[REDACTED]

ZENECA Diagnostics

# CF(12)m Test: Prototype Record Sheet

## B Tube [P8]

1x ARMS, 100µM dNTPs

Modifications since last prototype:

621+1/R117HC 3 bases longer at 5' end

Primer	F/R	Oligo ID	Mismatch	Length	Concn (µM)	Sequence
Apo B	F	S5890	CA -2	23	0.05	GAG CAC AGT ACG AAA AAC CAC CT
Apo B	R	S5891	CA -2	24	0.05	AAA CTT TTA CAG GGA TGG AGA ACG
ODC	F	0302-95	CT -2	30	0.30	AGA GGA TTA TCT ATG CAA ATC CTT GTA ACC
ODC	R	0303-95	AC -2	30	0.30	TCA ACT TCA CTA TCA AAA GTC ATC ATC TAG
DF N	R	0176-95	CT -2	30	0.50	GTA TCT ATA TTC ATC ATA GGA AAC ACC ACA
DF C	F	0177-95	-	30	0.50	GAC TTC ACT TCT AAT GAT GAT TAT GGG AGA
621 M	R	R029-96	GT -2	30	2.00	TGC CAT GGG GCC TGT GCA AGG AAG TAT TGA
R117H M	R	0179-95	CC -2	30	0.50	AGC CTA TGC CTA GAT AAA TCG CGA TAG ACT
621/R117H C	F	S7587	CT -8 TC -26 GA -27	37	1.50	GTT TCA CAT AGT GTA TGA CCC TCT ATA TAC ACT CAT T
R334W M	F	P1337	GG -2	30	0.20	CCT ATG CAC TAA TCA AAG GAA TCA TCC TGT
R334W C	R	0192-95	-	30	0.20	TTT GTT TAT TGC TCG AAG AGA GTC ATA CCA
G551D M	R	0190-95	TT -2	25	1.00	GCT AAA GAA ATT CTT GCT CGT TGT T
R553X M	R	0301-95	GG -2	60	2.00	GAC TGA CTG ACT GAC TGA CTC TGA CTG ACT TAT TCA CCT TGC TAA AGA AAT TCT TGC TGA
G551D/R553X C	F	0193-95	GG -20 GA -21	28	1.50	TAA AAT TGG AGC AAT GTT GTT TTT GAC C
R1162X M	F	0300-95	GT -2	30	0.20	TAT TTT TAT TTC AGA TGC GAT CTG TGA GTT
R1162X C	R	P5732	-	29	0.20	TTT TGC TGT GAG ATC TTT GAC AGT CAT TT

Operator

BUSIE WESTON

Date

Note: Oswel synthesis S7587 gave primer-dimer. It was replaced by Nikki's synthesis R072-96 which was fine.

**ZENECA** Diagnostics